

MARKED UP SPECIFICATION SHOWING AMENDMENTS

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Attorney's Docket No. [018422-000210] EXT-073 C1

Cross Reference to Related Applications on Page 1:

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of U.S. Patent Application Serial No. 09/336,609, filed June 18, 1999, which [The present application] claims priority to U.S. [Patent] Provisional Application Serial No. 60/090,063, filed June 19, 1998 and is related to U.S. Patent Application Serial No. 08/971,845, filed August 8, 1997, [herein both incorporated by reference.]; the entire disclosures of which are incorporated herein by reference.

First full paragraph on page 26:

Sequences of Oligonucleotides used:

4.5S probe (2nf): GGCACACGCGTCATCTGC (SEQ ID NO:[8]9)

5S probe (66ng): CCACACTACCATCGGCGCT (SEQ ID NO:[9]20)

First full paragraph on page 28:

Aliquots were thawed, 20% sodium dodecyl sulfate was added to a final concentration of 1.4% in a total volume of 15.6 μ l, and tubes were heated at 130°C for 10 minutes. Tubes were removed to room temperature for several minutes, and hybridization mix was added to a final volume of 20 μ l with the following final concentrations: 120 mM NaCl, 1 mM MgCl₂, 0.1 mM ZnCl₂, 22.5 mM Tris (pH 8), 22.5 mM boric acid, 0.5 mM aurin tricarboxylic acid, 8mM Na phosphate, and 50 nM of each of the alkaline phosphate-conjugated reporter probes, RP-1 (5'-alkaline phosphatase-GCUGCUUCCUUC (SEQ ID NO:[4]27); underlined bases represent 2'-O-methyl RNA nucleotides) and RP-2 (5'-alkaline phosphatase-GCUGCUUCCGUC (SEQ ID NO:14). These mixtures were warmed to 55°C for 10 minutes, then removed to room temperature and 4 μ l of loading buffer (50% glycerol, 0.2% xylene cyanole, 0.2% bromphenol blue) added. Half of each mixture was loaded onto a 5% polyacrylamide gel (89 mM Tris (pH 8.5), 27 mM phosphate buffer), made with 10 μ M of each of the following five acrydite-modified, 2'-O-methyl RNA capture probes, polymerized into the gel in a fashion similar to that described in Example III.

CP-1 5'-acrydite-TTTTTT-CGGACCUGACCUG (SEQ ID NO:15)

- CP-2 5'-acrydite-TTTTTT-AGGACCUGACAUG (SEQ ID NO:16)
CP-3 5'-acrydite-TTTTTT-CGGACCUGACCAG (SEQ ID NO:17)
CP-4 5'-acrydite-TTTTTT-CGGACCUGACAAG (SEQ ID NO:18)
CP-5 5'-acrydite-TTTTTT-CGGAUCUGACACG (SEQ ID NO:19)

The gel was run at 30° at 20 volts/cm for 30 minutes, rinsed in diethanolamine buffer (2.4 M diethanolamine, 1 mM MgCl₂, 0.1 mM ZnCl₂, pH 10) for 10 minutes, then AttoPhosTM chemifluorescent substrate (Boehringer-Mannheim) was added for 10 minutes. The reaction was stopped by the addition of 1M Na phosphate (pH 7.2) and the fluorescent signal was scanned on a Molecular Dynamics Fluorimager 595 (*see* Figure 7). All nine of the listed bacterial species were detected with this probe set.

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MARKED UP CLAIMS SHOWING AMENDMENTS

Claim 19:

19. (Amended) The method of claim 1, wherein the nucleic acid probe has a nucleotide sequence selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO:4); GCTGCTTCCTTCCGGACCTGAGTGAATACGTTCCCGGGCCT (SEQ ID NO:7); GCTGCTTCCTTCCGGACCTGACAAAAACGATAAACCAACCA (SEQ ID NO:8); GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:[14]21); CGGACCTGACCTG (SEQ ID NO:[15]22); AGGACCUGACAUG (SEQ ID NO:[16]23); CGGACCUGACCAG (SEQ ID NO:[17]24); CGGACCUGACAAG (SEQ ID NO:[18]25); and CGGAUCUGACACG (SEQ ID NO:[19]26).

Claim 41:

41. (Amended) The method of claim 20, wherein the gel-immobilized nucleic acid probe has the nucleotide sequence selected from the group consisting of: GCTGCTTCCTTCCGGACCTGAC (SEQ ID NO:2); GCTGCTTCCTTCCGGACCTGA (SEQ ID NO:3); GGCACACGCGTCATCTGC (SEQ ID NO:9); GCTGCTTCCTTC (SEQ ID NO: 4); GCTGCTTCCTTCCGGACCTGACCTGGTAAA (SEQ ID NO:11); GCTGCTTCCTTCCG (SEQ ID NO:5); GACCTGACCTGGTA (SEQ ID NO:6); GCTGCTTCCGTC (SEQ ID NO:[14]21); CGGACCTGACCTG (SEQ ID NO:[15]22); AGGACCUGACAUG (SEQ ID NO:[16]23); CGGACCUGACCAG (SEQ ID NO:[17]24); CGGACCUGACAAG (SEQ ID NO:[18]25); and CGGAUCUGACACG (SEQ ID NO:[19]26).